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(54) Title: USE OF BORANOCARBONATES FOR THE THERAPEUTIC DELIVERY OF CARBON MONOXIDE

(57) Abstract: Boranocarbonates are described for administration to a human or other mammal for delivery of carbon monoxide. The boranocarbonate is a compound or ion adapted to make CO available for physiological effect, and may be administered with a guanylate cyclase stimulant or stabilizer. The physiological effect may be stimulation of neurotransmission, vasodilation or smooth muscle relaxation.

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Therapeutic Delivery of Carbon Monoxide

FIELD OF THE INVENTION

The present invention relates to pharmaceutical compositions and compounds for the therapeutic delivery of carbon monoxide to humans and other mammals. Another use of the composition and compounds is in organ perfusion.

BACKGROUND OF THE INVENTION

Mammalian cells constantly generate carbon monoxide (CO) gas via the endogenous degradation of heme by a family of constitutive (HO-2) and inducible (HO-1) heme oxygenase enzymes [1,2]. First described as a putative neural messenger [3], CO is now regarded as a versatile signaling molecule having essential regulatory roles in a variety of physiological and pathophysiological processes that take place within the cardiovascular, nervous and immune systems. Indeed, CO produced in the vessel wall by heme oxygenase enzymes possesses vasorelaxing properties and has been shown to prevent vasoconstriction and both acute and chronic hypertension through stimulation of soluble guanylate cyclase [4-10]. Endogenous CO appears to modulate sinusoidal tone in the hepatic circulation [11], control the proliferation of vascular smooth muscle cells [12] and suppress the rejection of transplanted hearts [13]. The biological action of heme oxygenase-derived CO is substantiated by the pharmacological effects observed when this gas is applied exogenously to *in vitro* and *in vivo* systems. At concentrations ranging from 10 to 500 p.p.m., CO gas has been reported to mediate potent anti-inflammatory effects [14], prevent endothelial cell apoptosis [15], inhibit human airway smooth muscle cell proliferation [16] and promote protection against hyperoxic as well as ischemic lung injury [17,18]. In view of the pivotal role exerted by the heme oxygenase pathway in the control of cellular homeostasis [19] and the emerging pleiotropic properties

attributed to CO [20], it is conceivable that this diatomic gas could be used as a therapeutic tool for the treatment of vascular dysfunction and immuno-related disease states.

At present, three different approaches have been proposed for examining the therapeutic potential of CO: 1) direct administration of CO gas [20]; 2) use of pro-drugs (i.e. methylene chloride) which are catabolized by hepatic enzymes to generate CO [21]; and 3) transport and delivery of CO by means of specific CO carriers [22]. Some investigators have concentrated their efforts on the last strategic approach as it has been recently reported that certain transition metal carbonyls possess the ability to liberate CO under appropriate conditions and function as CO-releasing molecules (CO-RMs) in biological systems. In particular, it was shown that CO-RMs induce vessel relaxation in isolated aortic tissue and prevent coronary vasoconstriction as well as acute hypertension *in vivo* through specific mechanisms that can be simulated by activation of the HO-1/CO pathway [23]. Interestingly, the versatile chemistry of transition metals allows them to be effectively modified by coordinating biological ligands to the metal center in order to render the molecule less toxic, more water soluble and to modulate the release of CO. It has been recently reported that tricarbonylchloro(glycinato)ruthenium(II) (here called CORM-3), a newly synthesized water-soluble form of metal carbonyl that liberates CO *in vitro*, *ex-vivo* and *in vivo* biological models, protects myocardial cells and tissues against ischemia-reperfusion injury as well as cardiac allograft rejection [24,25]. Some of this work is published in International Patent Application WO 02/092075 (ref. 25).

In the case of CORM-3, the chloride and glycinate ligands are labile and their substitution with higher affinity ligands present in the cellular or plasma environment (i.e. glutathione) would appear to accelerate dissociation of CO from the metal center [27]. When added to a solution containing

myoglobin (Mb), the release of CO from CORM-3 is accelerated as 1 mole of CO per mole of compound is liberated within 1-2 min [24]. CORM-3 would, therefore, fall into a category of compounds that release CO very rapidly ("fast releasers") which can be ideal for several clinical applications in which CO acts as a signalling mediator (i.e. neurotransmission, acute hypertension, angina, ischemia-reperfusion); however, identifying compounds that release CO with a slow kinetics ("slow releasers") would implement the design of pharmaceuticals that could be more versatile in the treatment of certain chronic diseases (i.e. arthritis, inflammation, cancer, organ preservation; chronic hypertension; septic shock prevention of restenosis after balloon angioplasty, post-operative ileus) where the continuous and long-lasting effect of CO may be required.

An interesting example in the development of transition metal carbonyls that are used for medical applications not related to the therapeutic use of CO is represented by carbonyls specifically designed for radio-imaging technology. The recently described technetium(I) complex $[^{99m}Tc(OH_2)_3\text{-}(CO)_3]^+$ has attracted much interest as a precursor for technetium-99m radiopharmaceuticals [28]. A number of biomolecules, for example, peptides, scFv, and CNS receptor ligands, have already been labeled with technetium by this approach, demonstrating the potential of $[^{99m}Tc(OH_2)_3\text{-}(CO)_3]^+$ for radiopharmaceutical application [29]. This compound can be prepared in a single-step procedure from aqueous $[^{99m}TcO4]^-$ in the presence of CO and BH_4^- as a reducing agent [30].However, the published preparation of $[^{99m}Tc(OH_2)_3\text{-}(CO)_3]^+$ relying on gaseous carbon monoxide, is unsuitable for use in commercial radiopharmaceutical "kits". A recent study has reported the first commercially feasible preparation of $[^{99m}Tc(OH_2)_3\text{-}(CO)_3]^+$ in physiological media using a boron-based carbonylating agent, potassium boranocarbonate ($K_2[H_3BCO_2]$), which acts as a CO source and a reducing agent at the same time [31].

Boranocarbonates have been disclosed or suggested for physiological effects in the prior art. EP-A-34238 and EP-A-181721 describes anti-tumour and anti-hyperlipidemic activities of amine-carboxboranes. US-A-4312989 discloses use of amine boranes to inhibit the inflammation process. US-A-5254706 describes phosphite-borane compounds for anti-tumour, anti-inflammatory and hypolipidemic activity.

WO93/05795 discusses use of organic boron compounds effective against osteoporosis and suggests also anti-inflammatory, anti-hyperlipidemic and antineoplastic activity. The compounds disclosed are primarily of the amino-borane class, but Na_2BH_3COO is also tested. Hall et al., "Metal Based Drugs", Vol. 2, No. 1, 1995, describes anti-inflammatory activity of acyclic amine-carboxyboranes in rodents.

These documents reveal interest in the boron compounds either because of the possible effect of boron itself or because the amino-boranes are analogous to the natural α-amino acids.

SUMMARY OF THE INVENTION

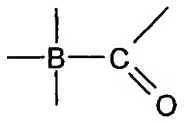
As exemplified by the experimental data detailed below, the present inventors have found that boranocarbonate compounds can be used to deliver CO to a physiological target so as to provide physiological effect.

Accordingly the present invention provides a pharmaceutical composition, intended for administration to a human or other mammal for delivery of carbon monoxide, comprising a boranocarbonate compound or ion adapted to make CO available for physiological effect and at least one pharmaceutically acceptable carrier.

Boranocarbonates are a group of compounds which can loosely be described as carboxylate adducts of borane and derivatives of borane. Boranocarbonates generally contain a group of the form $-COO^-$ or COOR (where R is H or another group) attached to the boron atom, so that they may be called

boranocarboxylates or carboxyboranes, but the term boranocarbonate seems to be preferred. The compound $K_2(H_3BCOO)$ and the related $K(H_3BCOOH)$ are described in reference 31, where the compound $K_2(H_3BCOO)$ is used for producing Tc carbonyls.

Thus typically a boranocarbonate has the molecular structure including the moiety



Preferred is the structure above with three hydrogen atoms attached to the boron (BH_3-CO-), since this is believed to facilitate CO release.

Also preferred are structures where a carboxylate group is attached to boron, i.e. $-COO^-$, -COOH-, -COOX where X may be any suitable esterifying group acceptable pharmaceutically.

Preferably the boranocarbonate compound in the pharmaceutical composition has an anion of the formula:

$BH_x(COQ)_yZ_z$

wherein:-

x is 1, 2 or 3

y is 1, 2 or 3

z is 0, 1 or 2

x + y + z = 4,

each Q is O^-, representing a carboxylate anionic form, or is OH, OR, NH_2, NHR, NR_2, SR or halogen, where the or each R is alkyl (preferably of 1 to 4 carbon atoms),

each Z is halogen, NH_2, NHR', NR'_2, SR' or OR' where the or each R' is alkyl (preferably of 1 to 4 carbon atoms).

Since this formula is analogous to the borano anion BH_4^-, the structure generally is an anion. It may be a divalent anion when one (COQ) is present as (COO^-). If the structure is an anion, a cation is required. Any physiologically suitable cation may be employed, particularly

a metal cation such as an alkali metal ion e.g. K^+ or Na^+ or an alkaline earth metal cation such as Ca^{++} or Mg^{++}. Alternatively non-metal cations might be employed, such as NR_4^+ where each R is H or alkyl (preferably of 1 to 4 carbon atoms) or PR_4^+ where R is alkyl (preferably of 1 to 4 carbon atoms). The cation may be selected in order to achieve a desired solubility of the compound.

Preferably y is 1. Preferably x is 3.

Preferably the boranocarbonate is soluble and is present in solution in a suitable solvent, e.g. an aqueous solvent, in the composition. Other possible solvents are ethanol, DMSO, DMF and other physiologically compatible solvents.

The boranocarbonates employed in the present invention vary in their ability to provide CO. The release of CO may be pH and temperature dependent. Lower pH causes more or faster release. Thus a range of compounds is available, for choice of a suitable release rate for a particular application. Slow release over a long period, of hours or days, can be achieved. Solutions can be provided containing dissolved CO, already released by the boranocarbonate. Alternatively, release of CO may be triggered by change of condition (e.g. pH or temperature) or by contact with another material, e.g. another solvent or aqueous physiological fluid such as blood or lymph, or even at a physiological delivery site.

Typically the pharmaceutical compositions of the present invention release CO such as to make it available to a therapeutic target in dissolved form. However, in some circumstances CO may be released directly to a non-solvent acceptor molecule.

It will be apparent that pharmaceutical compositions according to the present invention may be capable of delivering CO therapeutically through one or more of the above described modes of action.

The boranocarbonate compound may further comprise a targeting moiety, to facilitate release of CO at an

appropriate site. The targeting moiety is typically capable of binding a receptor on a particular target cell surface, in order to promote release of CO at the required site. The targeting moiety may be a part of a modulating ligand capable of binding to a receptor found on the surface of the target cells, or may be derived from another molecule, such as an antibody directed against a particular receptor, joined to the boranocarbonate molecule by a suitable linker.

The pharmaceutical compositions of the present invention typically comprise a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere unduly with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, subcutaneous, nasal, intramuscular, intraperitoneal, transdermal, transmucosal or suppository routes.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant or a slow-release polymer. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Pharmaceutically acceptable amounts of other solvents may also be included, in particular where they are required for dissolving the particular compound contained in the composition.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will typically be in the form of a parenterally acceptable solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art

are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. Delivery systems for needle-free injection are also known, and compositions for use with such systems may be prepared accordingly.

In pharmaceutical compositions intended for delivery by any route including but not limited to oral, nasal, mucosal, intravenous, cutaneous, subcutaneous and rectal the active substance may be micro encapsulated within polymeric spheres such that exposure to body fluids and subsequent CO release is delayed in time.

Administration is preferably in a prophylactically effective amount or a therapeutically effective amount (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

When formulating pharmaceutical compositions according to the present invention, the toxicity of the active ingredient and/or the solvent must be considered. The balance between medical benefit and toxicity should be taken into account. The dosages and formulations of the compositions will typically be determined so that the medical benefit provided outweighs any risks due to the toxicity of the constituents.

There is further provided a method of introducing CO to a mammal comprising the step of administering a pharmaceutical composition according to the present invention. CO is thought to act at least in part through stimulation or activation of guanylate cyclase. CO is thought to have functions as, inter alia, a neurotransmitter and a vasodilating agent. Accordingly there is provided a method of delivering CO to a mammal for stimulation of guanylate cyclase activity. There is further provided a method of delivering CO to a mammal for stimulating neurotransmission or vasodilation. However the present applicants do not wish to be bound by theory and do not exclude the possibility that CO operates by other mechanisms.

The heme oxygenase 1 (HO-1) pathway is thought to represent a pivotal endogenous inducible defensive system against stressful stimuli including UVA radiations, carcinogens, ischaemia-reperfusion damage, endotoxic shock and several other conditions characterised by production of oxygen free radicals (32,19,2). The protective effect of HO-1 is attributed to the generation of the powerful antioxidants biliverdin and bilirubin and the vasoactive gas CO. Expression of HO-1 has been linked with cardiac xenograft survival (33), suppression of transplant arteriosclerosis (34) and amelioration of post-ischemic myocardial dysfunction (35). HO-1 has also been directly implicated in the resolution phase of acute inflammation in rats (36). Other pathological situations, such as haemorrhagic shock in brain and liver as well as sepsis (37-39), are characterized by induction of the HO-1 gene, which seems to play a crucial role in counteracting the vascular dysfunction caused by these pathophysiological states. Increased generation of CO as a consequence of HO-1 induction markedly affects vessel contractility and diminishes acute hypertension in the whole organism (10,9). Exposure of animals to ambient air containing low concentrations of CO or transfection of the HO-1 gene results in protection against

hyperoxia-induced lung injury *in vivo*, a mechanism mediated by attenuation of both neutrophil inflammation and lung apoptosis (cell death) (17,40). Exogenous CO gas also has the ability to suppress pro-inflammatory cytokines and modulate the expression of the anti-inflammatory molecule, IL-10, both *in vitro* and *in vivo* (14). Therefore administration of CO in accordance with the invention may be used for treatment of any of these conditions, for modulation of inflammatory states and regression of other pathophysiological conditions including cancer.

Accordingly there is provided a method of introducing CO to a mammal comprising the step of administering a pharmaceutical composition according to the present invention, for treatment of hypertension, such as acute, pulmonary and chronic hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases such as asthma, rheumatoid arthritis and small bowel disease, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction and adult respiratory distress syndrome, and in procedures such as balloon angioplasty (to treat restenosis following balloon angioplasty) and aortic transplantation. For example, in balloon angioplasty it may be advantageous to make a local delivery of CO-releasing compound before and/or after the angioplasty. Alternatively, a stent may have a coating containing CO- releasing compounds.

The present invention also provides the use of a boranocarbonate compound or ion as herein described in the manufacture of a medicament for delivering CO to a physiological target, particularly a mammal, to provide a physiological effect, e.g. for stimulating neurotransmission or vasodilation, or for treatment of any of hypertension, such as acute, pulmonary and chronic hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related

diseases such as asthma, rheumatoid arthritis and small bowel disease, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, sickle cell anemia or sickle cell disease, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction and adult respiratory distress syndrome, and in procedures such as balloon angioplasty and aortic transplantation. Such medicaments may be adapted for administration by an oral, intravenous, subcutaneous, nasal, inhalatory, intramuscular, intraperitoneal, transdermal, transmucosal or suppository route.

In a further aspect, the invention provides a method of treatment of a mammal comprising stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or the treatment of any of hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, adult respiratory distress syndrome, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ, by administration of a boranocarbonate compound or ion adapted to make CO available for physiological effect. These are treatments associated with the action of CO.

Preferably, the method of treatment is stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or treatment of any of acute or chronic systemic hypertension, radiation damage, endotoxic shock, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis,

post-ischemic organ damage, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

More preferably, the method of treatment is stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or treatment of any of acute or chronic systemic hypertension, hyperoxia-induced injury, cancer by the pro-apoptotic effect of CO, transplant rejection, post-operative ileus, post-ischemic organ damage, angina, haemorrhagic shock, penile erectile dysfunction, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty or aortic transplantation.

Particularly, the method may be treatment of any of hyperoxia-induced injury, cancer by the pro-apoptotic effect of CO, transplant rejection, post-operative ileus, post-ischemic organ damage, angina, haemorrhagic shock, penile erectile dysfunction, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty or aortic transplantation.

By "smooth muscle relaxation" is meant treatment of conditions other than by vasodilation, such as chronic anal fissure, internal anal sphincter disease and anorectal disease.

More specific treatments to which the invention may be applied are the suppression of atherosclerotic legions following aortic transplantation, ischemic lung injury, prevention of reperfusion induced myocardial damage, and also to achieve the pro-apoptotic effects of CO (e.g. in cancer treatments).

The invention further provides use of the boranocarbonate compounds or ions here described in treatment, e.g. by perfusion, of a viable mammalian organ extracorporeally, e.g.

during storage and/or transport of an organ for transplant surgery. For this purpose, the boranocarbonate is in dissolved form, preferably in an aqueous solution. The viable organ may be any tissue containing living cells, such as a heart, a kidney, a liver, a skin or muscle flap, etc.

For example, isolated organs e.g. extracorporeal organs or in situ organs isolated from the blood supply can be treated. The organ may be, for example, a circulatory organ, respiratory organ, urinary organ, digestive organ, reproductive organ, neurological organ, muscle or skin flap or an artificial organ containing viable cells. In particular, the organ may be a heart, lung, kidney or liver. However, the body tissue which is treatable are not limited and may be any human or mammal body tissue whether extracorporeal or in-situ in the body. It is further believed that the compositions of the invention here described are useful to deliver CO to an extracorporeal or isolated organ so as to reduce ischaemic damage of the organ tissue.

Within the present invention, the boranocarbonates here described can be used in combination with a guanylate cyclase stimulant or stabilizer to deliver CO to a physiological target so as to provide an improved physiological effect.

The pharmaceutical preparation may contain the boranocarbonate and the guanylate cyclase stimulant/stabilizer in a single composition or the two components may be formulated separately for simultaneous or sequential administration.

Thus the present invention provides a method of introducing CO to a mammal as a therapeutic agent comprising:

a) administering a boranocarbonate which makes available CO suitable for physiological effect; and

b) administering a guanylate cyclase stimulant or stabiliser.

In this aspect, the method is particularly applicable to treatment of acute or chronic systemic hypertension, pulmonary

hypertension, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure, chronic anal fissure, internal anal sphincter disease, anorectal disease, and ulcerative colitis or for treatment in balloon angioplasty or aortic transplantation.

Preferably, the stabilizer/stimulant is administered first followed by the boranocarbonate but this order may be reversed.

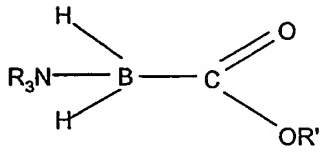
The guanylate cyclase stabilizer/stimulant compound may be any compound which stimulates production of guanylate cyclase or which stabilizes guanylate cyclase, in particular the active form of guanylate cyclase. A single compound can be used or a combination of compounds can be used either for simultaneous or sequential administration, i.e. the various aspects include/use at least one guanylate cyclase stimulant/stabilizer.

Examples include 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1), 4 pyrimidinamine-5-cyclopropyl-2-[1-[(2-fluorophenyl)methyl]-1H-pyrazolo[3,4-b]pyridin-3-yl] (BAY 41-2272), BAY 50-6038 (ortho-PAL), BAY 51-9491 (meta PAL), and BAY 50-8364 (para PAL). The structures of ortho-, meta- and para- PAL are shown in Figure 9 attached. These compounds have been found to bind to an activation site on the guanylate cyclase and any other compounds that similarly bind to the site may be useful as the guanylate cyclase stabilizer/ stimulant. Also useful are NO donors and 1-benzyl-3-(3^1-ethoxycarbonyl)phenyl-indazole, 1-benzyl-3-(3^1-hydroxymethyl)phenyl-indazole, 1-benzyl-3-(5^1-diethylaminomethyl)-furyl-indazole, 1-benzyl-3-(5^1-methoxymethyl)furyl-indazole, 1-benzyl-3-(5^1-hydroxymethyl)furyl-6-methyl-indazole, 1-benzyl-3-(5^1-hydroxymethyl)-furyl-indazol-benzyl-3-(5^1-hydroxymethyl)-furyl-indazole, 1-benzyl-3-(5^1-hydroxymethyl)-furyl-6-fluoro-

indazole, 1-benzyl-3-(5^1-hydroxymethyl)-furyl-6-methoxy-indazole, and 1-benzyl-3-(5^1-hydroxymethyl)-furyl-5,6-methylenedioxoindazole or pharmaceutically acceptable salts thereof.

For reasons relating to prior patent filings and for proprietary reasons, the present applicants may wish to exclude use of the following two compounds from the protection given to the present invention in any of its aspects as claimed:-

I. K_2 (H_3BCOO)

II. 

where R, R' = H, alkyl, perfluoroalkyl.

Therefore this exclusion is now optionally and provisionally made.

Throughout this application, references to medical treatment are intended to include both human and veterinary treatment, and references to pharmaceutical compositions are accordingly intended to encompass compositions for use in human or veterinary treatment.

Experimental data illustrating the present invention will now be described.

In the accompanying drawings, Figs 1 to 8 are graphs showing results of the experiments of Examples 1 to 8 below. Fig. 9 is chemical formulae mentioned above. Figs 10 and 11 are graphs showing results of Examples 9 and 10 below.

EXAMPLES 1 TO 8

Reagents

Tricarbonylchloro(glycinato)ruthenium(II) ([$Ru(CO)_3Cl$(glycinate)] or CORM-3) was synthesized as previously described by Clark and collaborators [24]. Disodium boranocarbonate ($Na_2[H_3BCO_2]$, indicated here as "CORM-A1") was synthesized as previously described by Alberto and collaborators [31]. Sodium borohydride ($NaBH_4$) and all other reagents were from Sigma Chemicals (Poole, Dorset).

Preparation of inactive CORM-A1 and its use as negative control

The chemistry of boranocarbonate in aqueous solution has been previously described [31]. This compound is relatively stable in distilled water at basic pH. The compound starts to release CO as the pH moves towards more physiological conditions (pH=7.4) and the rate of CO release is greatly accelerated at acidic pH. Based on this evidence, we generated an inactive form of CORM-A1 (iCORM-A1) by reaction of the compound with acid. Specifically, a small aliquot (10 μl) of concentrated hydrochloric acid (10 M) was added to 1 ml of CORM-A1 in water (100 mM final concentration). The reaction resulted in a rapid evolution of a gas (presumably CO); the solution was then bubbled with a stream of nitrogen in order to remove the residual CO gas eventually dissolved. Aliquots of this solution were used as a negative control of CORM-A1 in the experiments conducted to quantify the release of CO (i.e. Mb assay) as well as the biological efficacy (i.e. vessel relaxation). Since boron is a component of CORM-A1 and because borohydride could be formed during the liberation of CO from CORM-A1 in aqueous solution, sodium borohydride ($NaBH_4$) was also utilized as a negative control in some experiments.

Detection of CO release

The release of CO from CORM-A1 was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (Mb) to carbonmonoxy myoglobin (MbCO) by a method previously described [23]. The amount of MbCO formed was quantified by measuring the absorbance at 540 nm (extinction coefficient = 15.4 M^{-1} cm^{-1}) over time at 37 °C. Myoglobin solutions (approximately 50 μmol/L final concentration) were prepared fresh by dissolving the protein in 0.04 M phosphate buffer (pH=7.4). Sodium dithionite (0.1 %) was added to convert the oxidized myoglobin to its reduced form prior to each reading. Some experiments were also conducted using Mb at pH=5.5 or at room temperature (RT) in order to examine the kinetic of CO release from CORM-A1 under different chemical and physical conditions.

Isolated aortic ring preparation: studies on vessel relaxation

Transverse ring sections of thoracic aorta were isolated from male Lewis rats and suspended under a 2 g tension in an organ bath containing oxygenated Krebs-Henseleit buffer at 37 °C in a manner previously described [10]. The relaxation response to CORM-A1 (40, 80 and 160 μM) was assessed in aortic rings pre-contracted with phenylephrine (3 μM). Control rings were similarly treated by adding equal doses of the inactive compound (iCORM-A1) or sodium borohydride ($NaBH_4$) to the organ bath. Experiments were also conducted by comparing the effect of CORM-A1 and CORM-3 on vessel relaxation over time.

Example 1. **Conversion of myoglobin (Mb) to carbon monoxide myoglobin (MbCO) by CO gas.**

Myoglobin (Mb) in its reduced state displays a characteristic spectrum with a maximal absorption peak at 555 nm (see Figure 1, dotted line). When a solution of Mb (50 μM) is bubbled for

1 min with CO gas (1%), a rapid conversion to carbon monoxide myoglobin (MbCO) is observed. As shown in Figure 1, MbCO displays a characteristic spectrum with two maximal absorption peaks at 540 and 576 nm, respectively (solid line). This method has been previously developed to monitor and determine the amount of CO released from CO-RMs [23] and can be used to examine how various conditions such as different pHs and temperatures can affect the kinetics of CO release (see Examples 4).

Example 2. **Conversion of myoglobin (Mb) to carbon monoxide myoglobin (MbCO) by CORM-A1.**

Addition of CORM-A1 (60 μM) to a solution containing reduced Mb (pH=7.4, temp. = 37 °C) resulted in a gradual formation of MbCO over time. As shown in Figure 2, a spectrum typical of reduced Mb (filled square) is converted to a spectrum characteristic of MbCO after 210 min incubation (inverted open triangle). The trace with asterisks shows the spectrum of MbCO when Mb is saturated with CO gas (positive control) as described in Materials and Methods.

Example 3. **Kinetics of CO release from CORM-A1 at room temperature.**

The amount of MbCO formed after addition of CORM-A1 to the Mb solution can be quantified by measuring the absorbance at 540 nm knowing the extinction coefficient for MbCO (ε = 15.4 M^{-1} cm^{-1}). CORM-A1 at three different concentrations was added to a solution containing Mb at room temperature and the formed MbCO was calculated over time. Non-linear regression analysis using one phase exponential association (GraphPad Prism) resulted in the best fitting of the three curves (r^2>0.99). As shown in Figure 3, the amount of MbCO formed from CORM-A1 increases with a defined kinetic in a concentration-dependent manner. The calculated Y_{max} for each plot (16.7±1.2, 33.1±1.4 and 48.2±2.5) was in very good agreement with the three

concentrations of CORM-A1 used (15.6, 31.1 and 46.7 μM, respectively). This indicates that the reaction leading to the formation of CO from CORM-A1 in aqueous solution goes to completion over time and that one mole of CO per mole of compound is liberated. From the fitted curves the average half-life of CORM-A1 at room temperature is 112±3 min.

Example 4. **Effects of temperature and pH on the rate of CO release from CORM-A1.**

The rate of CO release from CORM-A1 was examined at different pHs and temperatures. CORM-A1 (60 μM) was added to the Mb solution under three different conditions: 1) at room temperature (RT) and pH=7.4; 2) at 37 °C and pH=7.4; and 3) at 37 °C and pH = 5.5. The concentration of MbCO was calculated at different time points and non-linear regression analysis was used to obtain the best fitting of the three curves as described in example 3. As shown in Figure 4, the rate of CO release from CORM-A1 is significantly accelerated by increasing the temperature as well as by decreasing the pH. Specifically, it can be calculated that the half-life of CORM-A1 is 104 min at RT/pH=7.4 (triangles), 18.5 min at 37 °C/pH=7.4 (diamonds) and 1.2 min at 37 °C/pH=5.5 (squares).

Example 5. **Comparison between CORM-A1 and its inactive form (iCORM-A1) on their ability to liberate CO.**

As described in the Materials and Methods section, CO is rapidly lost when CORM-A1 is added to acidic solutions. This step allows the generation of an inactive compound (iCORM-A1) that could be used as an ideal negative control for testing the biological activity of these molecules. To verify that iCORM-A1 has effectively lost its full ability to release CO, the compound (60 μM) was added to a solution containing Mb (50 μM) at pH=7.4/RT and the MbCO formed over time was calculated. As shown in Figure 5, iCORM-A1 (circles) is incapable of generating any detectable MbCO suggesting that the compound

has been fully inactivated. The effect of CORM-A1 (squares) on MbCO formation is shown for comparison.

Example 6. **Comparison between CORM-A1 and CORM-3 in their ability to elicit vasorelaxation.**

CORM-3 ($[Ru(CO)_3Cl(glycinate)]$) has been shown to promote a rapid and significant relaxation in isolated vessels and this effect has been demonstrated to be mediated by CO [27]. It is also known from recent works that the liberation of CO from CORM-3 to Mb or in biological systems occurs very rapidly (approximately 5 min) [24,27], which is in agreement with the prompt pharmacological effects observed in isolated vessels. In the case of CORM-A1, the release of CO at physiological pH is slower (18.4 min) as shown in example 5. Thus, it is expected that the pharmacological action of CORM-A1 would reflect its biochemical behaviour. Indeed, as shown in Figure 6, CORM-A1 (80 μM) caused a much slower effect on relaxation compared to CORM-3 (80 μM). Specifically, CORM-3 (solid line) added to isolated aortic rings pre-contracted with phenylephrine (Phe) promoted a 75% relaxation within 4-5 min whereas CORM-A1 (dashed line) caused a gradual vasorelaxation which was maximal (96%) 33 min following addition of the compound to the organ bath.

Example 7. **Concentration-dependent effect of CORM-A1 on vasorelaxation**

Pre-contracted aortic rings were treated with increasing concentrations of CORM-A1 (40, 80 and 160 μM) and the percentage of vasorelaxation was calculated at different time points. As shown in Figure 7, CORMA-1 caused a significant relaxation over time in a concentration-dependent manner. For instance, it can be seen from the graph that after 10 min, the percentage of relaxation elicited by the different concentrations of CORM-A1 compared to control was as follows: 21.0±2.3% with 40 μM CORM-A1, 40.2±3.4% with 80 μM CORM-A1 and

74.9±1.8% with 160 μM CORM-A1. The data are represented as the mean±S.E.M. of 6 independent experiments for each group.

Example 8. **The vasorelaxant properties of CORM-A1 are mediated by CO**

Pre-contracted aortic rings were treated with 80 μM CORM-A1, iCORM-A1 (the inactive compound) or $NaBH_4$, which was used as an additional negative control (see Materials and Methods for details). As shown in Figure 8, only CORM-A1 promoted a gradual and profound vasorelaxation whereas both iCORM-A1 and $NaBH_4$ were totally ineffective. These results clearly suggest that CO liberated from CORM-A1 is directly responsible for the observed pharmacological effect. The data are represented as the mean±S.E.M. of 6 independent experiments for each group.

Examples 9 and 10.

Stock solutions of sodium boranocarbonate (CORM-A1, 100 mM) were prepared by solubilizing the compound in distilled water prior to the experiment. 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1) was purchased from Sigma-Aldrich (Poole, Dorset) and prepared in dimethyl sulfoxide (DMSO). All data are expressed as mean ± s.e.m. Differences between the groups analysed were assessed by the Student's two-tailed *t*-test, and an analysis of variance (ANOVA) was performed where more than two treatments were compared. Results were considered statistically significant at $P<0.05$.

Isolated aortic ring preparation: studies on vessel relaxation

Transverse ring sections of thoracic aorta were isolated from male Lewis rats and suspended under a 2 g tension in an organ bath containing oxygenated Krebs-Henseleit buffer at 37 °C in a manner previously described [10]. The relaxation response to CORM-A1 (20 μM) in the presence or absence of YC-1 (1 μM final concentration) was assessed over time in aortic rings pre-contracted with phenylephrine (1 μmol/L). YC-1 was added to

the isolated rings 30 min prior to contraction with phenylephrine.

Animal studies: effect of CORM-A1 and YC-1 on blood pressure Lewis rats (280-350 g) were anaesthetised by intramuscular injection of 1 ml/kg Hypnorm. Specially designed femoral artery and venous catheters were then surgically implanted and mean arterial pressure (MAP) monitored continuously using a polygraph recorder in a manner previously described [23]. The effect of CORM-A1 on mean arterial pressure (MAP) over time was assessed following an intravenous (i.v.) injection of 50 μmol kg^{-1}. Similar experiments were conducted by administering YC-1 (1.2 μmol kg^{-1}, i.v.) to animals 5 min prior to the bolus addition of CORM-A1. Control experiments using YC-1 alone were also performed.

Example 9. **Effect of CORM-A1 and YC-1 on aortic vasorelaxation** Pre-contracted aortic rings were treated with CORM-A1 and the percentage of vasorelaxation was calculated at different time points. As shown in Figure 10, 20 μM CORMA-1 caused 13±4.9% relaxation after 20 min; interestingly, a more pronounced and significant relaxation response (61±6.2%) was detected after pre-treatment of vessels with YC-1 (1 μM). Note that in control vessels pre-treated with YC-1 alone and contracted with phenylephrine there was only a minor relaxation response over time (2.8±1.1% after 20 min). The relaxation response of vessels pre-treated with YC-1 was also very significant at 1 μM and 10 μM CORM-A1 (35±9.8% and 51±3.3%, respectively). The data are represented as the mean±s.e.m. of 6 independent experiments for each group. *P<0.05 vs. CORM-A1 alone or YC-1 alone.

Example 10. **Effect of CORM-A1 and CORM-3 on mean arterial pressure.** Femoral artery and venous catheters were surgically

implanted into anesthetized Lewis rats and blood pressure continuously monitored as previously described by us [23]. The effect of CORM-A1 and YC-1 on mean arterial pressure (MAP) *in vivo* is represented in Figure 11. The compounds were injected intravenously as a bolus at a final concentration of 50 μmoles/kg for CORM-A1 and 1.2 μmol kg^{-1} for YC-1. When the two compounds were given in combination, YC-1 was administered 10 min prior to CORM-A1 injection. As shown, CORM-A1 produced a gradual and sustained decrease in MAP over time; for instance, 60 min after CORM-A1 injection MAP decreased by 6.3±1.5 mmHg from the initial baseline value. Injection with YC-1 alone also produced an effect on blood pressure; however, the decrease in MAP was only transient, reaching a maximum of 5.5±1.0 mmHg after 10 min and returning to basal levels 50 min after injection. Interestingly, the combination of CORM-A1 and YC-1 produced a synergistic effect resulting in a rapid and profound hypotension. In fact, MAP significantly decreased by 16.1±5.6 mmHg after 10 min and remained at this level for the rest of the experiment. The data are represented as the mean±s.e.m. of 5 independent experiments for each group. *$P<0.05$ vs. baseline (-10 min); † $P<0.05$ vs. CORM-A1 alone or YC-1 alone.

The present invention therefore provides water-soluble compounds which are useful as CO carriers which can have selectable chemical properties, enabling novel therapeutic approaches based on CO delivery. This offers significant advantages over inhalation of CO as it may circumvent the problems related to the systemic effects of CO gas on oxygen transport and delivery. Moreover, the design of stable compounds with "fast" or "slow" kinetics of CO release that could target selective organs and affect only a restricted area of the body is highly feasible. One application for the use of water-soluble compounds is in conditions where Co needs to be applied locally. For instance, in order to protect vascular tissues during balloon angioplasty and prevent blood

vessel restenosis, CO-providing compounds may be applied to vessels prior to the angioplasty procedure. Alternatively, vascular stents may be covered with specific boranocarbonate compounds that have the ability to release CO slowly to the injured vessels and inhibit smooth muscle cell proliferation. Compounds whose kinetic of CO release is affected by temperature could also be used *ex-vivo* as an adjuvant to preservation solutions that are commonly employed to store organs prior to transplantation. The protective role of HO-1 against organ rejection has been extensively reported and the concept of treating the organ(s) rather than the recipient(s) will have much benefit in the clinical setting of transplantation.

References

1. Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. *J Biol Chem.* 1969;244:6388-6394.

2. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol.* 1997;37:517-554.

3. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science.* 1993;259:381-384.

4. Sacerdoti D, Escalante B, Abraham NG, McGiff JC, Levere RD, Schwartzman ML. Treatment with tin prevents the development of hypertension in spontaneously hypertensive rats. *Science.* 1989;243:388-390.

5. Marks GS, Brien JF, Nakatsu K, McLaughlin BE. Does carbon monoxide have a physiological function? *Trends Pharmacol Sci.* 1991;12:185-188.

6. Coceani F, Kelsey L, Seidlitz E, Marks GS, McLaughlin BE, Vreman HJ, Stevenson DK, Rabinovitch M, Ackerley C. Carbon monoxide formation in the ductus arteriosus in the lamb: implications for the regulation of muscle tone. *Br J Pharmacol.* 1997;120:599-608.

7. Johnson RA, Colombari E, Colombari DSA, Lavesa M, Talman WT, Nasjletti A. Role of endogenous carbon monoxide in central regulation of arterial pressure. *Hypertension.* 1997;30:962-967.

8. Wang R, Wang ZZ, Wu LY. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol.* 1997;121:927-934.

9. Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, Winslow RM. Heme oxygenase-1-derived carbon monoxide contributes to the suppression of acute hypertensive responses *in vivo*. *Circ Res*. 1998;83:568-577.

10. Sammut IA, Foresti R, Clark JE, Exon DJ, Vesely MJJ, Sarathchandra P, Green CJ, Motterlini R. Carbon monoxide is a major contributor to the regulation of vascular tone in aortas expressing high levels of haeme oxygenase-1. *Br J Pharmacol*. 1998;125:1437-1444.

11. Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, Ishimura Y. Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. *J Clin Invest*. 1995;96:2431-2437.

12. Morita T, Mitsialis SA, Koike H, Liu YX, Kourembanas S. Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle cells. *J Biol Chem*. 1997;272:32804-32809.

13. Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AM, Bach FH, Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol*. 2001;166:4185-4194.

14. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med*. 2000;6:422-8.

15. Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP. Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. *J Exp Med*. 2000;192:1015-1026.

16. Song R, Mahidhara RS, Liu F, Ning W, Otterbein LE, Choi AM. Carbon monoxide inhibits human airway smooth muscle cell proliferation via mitogen-activated protein kinase pathway. *Am J Respir Cell Mol Biol.* 2002;27:603-610.

17. Otterbein LE, Mantell LL, Choi AMK. Carbon monoxide provides protection against hyperoxic lung injury. *Am J Physiol.* 1999;276:L688-L694.

18. Fujita T, Toda K, Karimova A, Yan SF, Naka Y, Yet SF, Pinsky DJ. Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. *Nat Med.* 2001;7:598-604.

19. Foresti R, Motterlini R. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. *Free Rad Res.* 1999;31:459-475.

20. Otterbein LE. Carbon monoxide: innovative anti-inflammatory properties of an age-old gas molecule. *Antioxid Redox Signal.* 2002;4:309-319.

21. Chauveau C, Bouchet D, Roussel JC, Mathieu P, Braudeau C, Renaudin K, Tesson L, Soulillou JP, Iyer S, Buelow R, Anegon I. Gene transfer of heme oxygenase-1 and carbon monoxide delivery inhibit chronic rejection. *Am J Transplant.* 2002;2:581-592.

22. Motterlini R, Foresti R, Green CJ. Studies on the development of carbon monoxide-releasing molecules: potential applications for the treatment of cardiovascular dysfunction. In: Carbon Monoxide and Cardiovascular Functions. Wang R, ed. 2002. CRC Press, Boca Raton, Florida.

23. Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, Green CJ. Carbon monoxide-releasing molecules:

characterization of biochemical and vascular activities. *Circ Res.* 2002;90:E17-E24.

24. Clark JE, Naughton P, Shurey S, Green CJ, Johnson TR, Mann BE, Foresti R, Motterlini R. Cardioprotective actions by a water-soluble carbon monoxide-releasing molecule. *Circ Res.* 2003;93:e2-e8.

25. Mann BE, Motterlini R. Therapeutic delivery of carbon monoxide. *PCT.* 2002;WO 02092075.

26. Johnson TR, Mann BE, Clark JE, Foresti R, Green CJ, Motterlini R. Metal carbonyls: a new class of pharmaceuticals? *Angew Chem Int Ed Engl.* 2003;In press.

27. Motterlini R, Mann BE, Johnson TR, Clark JE, Foresti R, Green CJ. Bioactivity and pharmacological actions of carbon monoxide-releasing molecules. *Curr Pharm Des.* 2003;In press.

28. Waibel R, Alberto R, Willuda J, Finnern R, Schibli R, Stichelberger A, Egli A, Abram U, Mach JP, Pluckthun A, Schubiger PA. Stable one-step technetium-99m labeling of His-tagged recombinant proteins with a novel Tc(I)-carbonyl complex. *Nat Biotechnol.* 1999;17:897-901.

29. Egli A, Alberto R, Tannahill L, Schibli R, Abram U, Schaffland A, Waibel R, Tourwe D, Jeannin L, Iterbeke K, Schubiger PA. Organometallic 99mTc-aquaion labels peptide to an unprecedented high specific activity. *J Nucl Med.* 1999;40:1913-1917.

30. Alberto R, Schibli R, Egli A, Schubiger AP, Abram U, Kaden TA. A novel organometallic aqua complex of technetium for the labeling of biomolecules: Synthesis of [Tc-99m(OH2)(3)(CO)(3)](+) from [(TcO4)-Tc-99m](-) in aqueous solution and its reaction with a bifunctional ligand. *J Am Chem Soc.* 1998;120:7987-7988.

31. Alberto R, Ortner K, Wheatley N, Schibli R, Schubiger AP. Synthesis and properties of boranocarbonate: a convenient in situ CO source for the aqueous preparation of [(99m)Tc(OH(2))3(CO)3]+. *J Am Chem Soc.* 2001;123:3135-3136.

32. Abraham NG, Drummond GS, Lutton JD, Kappas A. The biological significance and physiological role of heme oxygenase. Cell Physiol Biochem 1996;6:129-68.

33. Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RP, Choi AM, Poss KD, et al. Expression of heme oxygenase-1 can determine cardiac xenograft survival. Nature Med 1998;4:1073-7.

34. Hancock WW, Buelow R, Sayegh MH, Turka LA. Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. Nature Med 1998;4:1392-6.

35. Clark JE, Foresti R, Sarathchandra P, Kaur H, Green CJ, Motterlini R. Heme oxygenase-1-derived bilirubin ameliorates post-ischemic myocardial dysfunction. Am J Physiol Heart Circ Physiol 2000;278:H643-51.

36. Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of inflammatory response. Nature Med 1996;2:87-90.

37. Bauer M, Pannen BHJ, Bauer I, Herzog C, Wanner GA, Hanselmann R, Zhang JX, Clemens MG, Larsen R. Evidence for a functional-link between stress-response and vascular control in hepatic portal circulation. Am J Physiol 1996;271:G929-35.

38. Fukuda K, Panter SS, Sharp FR, Noble LJ. Induction of heme oxygenase-1 (HO-1) after traumatic brain injury in the rat. Neurosci Lett 1995;199:127-30.

39. Yet SF, Pellacani A, Patterson C, Tan L, Folta SC, Foster L, Lee WS, Hsieh CM, Perrella MA. Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. J Biol Chem 1997;272:4295-301.

40. Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, Choi AMK. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. J Clin Invest 1999;103:1047-54.

CLAIMS

1. Use of a boranocarbonate compound or ion in the manufacture of a medicament, for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or for the treatment of any of hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, adult respiratory distress syndrome, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

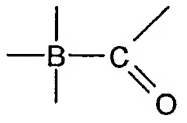
2. Use according to claim 1 wherein the medicament is for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or for the treatment of any of acute or chronic systematic hypertension, radiation damage, endotoxic shock, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

3. Use according to claim 1 wherein the medicament is for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or for the treatment of any of acute or chronic systematic hypertension, hyperoxia-induced injury, cancer by the pro-apoptotic effect of CO, transplant rejection, post-operative ileus, post-ischemic organ damage, angina, haemorrhagic shock,

penile erectile dysfunction, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty or aortic transplantation.

4. Use according to any one of claims 1, 2 and 3 wherein the medicament is suitable for administration by an oral, intravenous, subcutaneous, nasal, inhalatory, intramuscular, intraperitoneal, transdermal, transmucosal or suppository route.

5. Use according to any one of claims 1 to 4 wherein the molecular structure of the boranocarbonate compound or ion includes the moiety



6. Use according to claim 5 wherein the boranocarbonate compound or ion includes the moiety BH_3-CO-.

7. Use according to claim 5 or 6 wherein the boranocarbonate is a compound or anion of the formula:

$BH_x(COQ)_yZ_z$

wherein:-

x is 1, 2 or 3

y is 1, 2 or 3

z is 0, 1 or 2

x + y + z = 4,

each Q is O^-, representing a carboxylate anionic form, or is OH, OR, NH_2, NHR, NR_2, SR or halogen, where the or each R is alkyl (preferably of 1 to 4 carbon atoms),

each Z is halogen, NH_2, NHR', NR'_2, SR' or OR' where the or each R' is alkyl (preferably of 1 to 4 carbon atoms).

8. Use according to claim 7 wherein z is 0.

9. Use according to claim 8 or 9 where y is 1.

10. Use according to claim 7 where x is 3.

11. Use according to any one of claims 7 to 10 where the boranocarbonate is an anion, with at least one Q in the form of O^- or OR, and the composition includes at least one metal cation.

12. Use according to claim 11 wherein the or each metal cation is an alkali metal cation or an alkaline earth metal cation.

13. Use according to claim 12 wherein the boranocarbonate is $Na_2(H_3BCO_2)$.

14. Use according to any one of claims 1 to 13 wherein the medicament further includes a guanylate cyclase stimulant or stabilizer.

15. Use according to claim 14 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

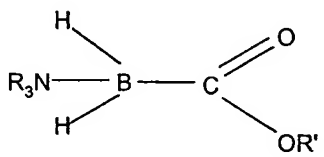
16. Use according to claim 14 or 15 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

17. Use according to any one of claims 14 to 16 wherein the medicament is adapted for one of simultaneous and sequential administration of the boranocarbonate compound or ion and the guanylate cyclase stimulant or stabilizer..

18. Use according to any one of claims 1 to 17 wherein the boranocarbonate compound or ion is other than

I. K_2 (H_3BCOO)

II.



where R, R' = H, alkyl, perfluoroalkyl.

19. Method of treatment of a mammal comprising stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or the treatment of any of hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, adult respiratory distress syndrome, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ, by administration of a boranocarbonate compound or ion adapted to make CO available for physiological effect.

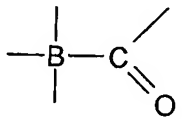
20. Method according to claim 19 comprising stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or treatment of any of acute or chronic systemic hypertension, radiation damage, endotoxic shock, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis, or treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

21. Method according to claim 19 comprising stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or treatment of any of acute or chronic systemic hypertension, hyperoxia-induced injury, cancer by the pro-apoptotic effect of CO, transplant rejection, post-operative ileus, post-ischemic organ damage, angina, haemorrhagic shock, penile erectile dysfunction, hepatic cirrhosis, cardiac hypertrophy, heart failure and

ulcerative colitis, or treatment in balloon angioplasty or aortic transplantation.

22. Method according to any one of claims 19, 20 or 21 wherein including administration by an oral, intravenous, subcutaneous, nasal, inhalatory, intramuscular, intraperitoneal, transdermal, transmucosal or suppository route.

23. Method according to any one of claims 19 to 22 wherein the molecular structure of the boranocarbonate compound or ion includes the moiety



24. Method according to claim 23 wherein the boranocarbonate compound or ion includes the moiety BH_3-CO-.

25. Method according to claim 23 or 24 wherein the boranocarbonate is a compound or anion of the formula:

$BH_x(COQ)_yZ_z$

wherein:-

x is 1, 2 or 3

y is 1, 2 or 3

z is 0, 1 or 2

x + y + z = 4,

each Q is O^-, representing a carboxylate anionic form, or is OH, OR, NH_2, NHR, NR_2, SR or halogen, where the or each R is alkyl (preferably of 1 to 4 carbon atoms),

each Z is halogen, NH_2, NHR', NR'_2, SR' or OR' where the or each R' is alkyl (preferably of 1 to 4 carbon atoms).

26. Method according to claim 25 wherein z is 0.

27. Method according to claim 25 or 26 where y is 1.

28. Method according to claim 25 where x is 3.

29. Method according to any one of claims 25 to 28 where the boranocarbonate is an anion, with at least one Q in the form of O^- or OR, and the composition includes at least one metal cation.

30. Method according to claim 29 wherein the or each metal cation is an alkali metal cation or an alkaline earth metal cation.

31. Method according to claim 29 wherein the boranocarbonate is $Na_2(H_3BCO_2)$.

32. Method according to any one of claims 19 to 31 wherein the medicament further includes a guanylate cyclase stimulant or stabilizer.

33. Method according to claim 32 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

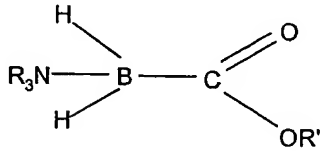
34. Method according to claim 32 or 33 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

35. Method according to any one of claims 32 to 34 comprising simultaneous or sequential administration of the boranocarbonate compound or ion and the guanylate cyclase stimulant or stabilizer.

36. Use according to any one of claims 19 to 35 wherein the boranocarbonate compound or ion is other than

I. K_2 (H_3BCOO)

II.



where R, R' = H, alkyl, perfluoroalkyl.

37. A method of treating a viable mammalian organ extracorporeally or an isolated mammalian organ, comprising

contacting the organ with a pharmaceutical composition comprising a boranocarbonate compound or ion adapted to make CO available for physiological effect.

38. A method according to claim 37 wherein the boranocarbonate compound or ion is as defined in any one of claims 5 to 13.

39. Method according to 38 or 39 wherein the composition further includes a guanylate cyclase stimulant or stabilizer.

40. Method according to claim 39 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

41. Method according to claim 39 or 40 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

42. A medical or veterinary implant carrying, in a form releasable at the implant site, a boranocarbonate compound or ion adapted to make CO available for physiological effect.

43. An implant according to claim 38 wherein the boranocarbonate compound or ion is as defined in any one of claims 5 to 13.

44. An implant according to 42 or 43 wherein the medicament further includes a guanylate cyclase stimulant or stabilizer.

45. An implant according to claim 44 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

46. An implant according to claim 44 or 45 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

47. A method of introducing CO to a mammal as a therapeutic agent comprising:

a) administering a boranocarbonate which makes available CO suitable for physiological effect; and

b) administering a guanylate cyclase stimulant or stabiliser.

48. A method according to claim 47, which is for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent,

or for the treatment of any of hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, adult respiratory distress syndrome, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

49. A method according to claim 47, which is for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or for the treatment of any of acute or chronic systematic hypertension, radiation damage, endotoxic shock, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, post-operative ileus, arteriosclerosis, post-ischemic organ damage, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty, aortic transplantation or survival of a transplanted organ.

50. A method according to claim 47, which for the stimulation of neurotransmission, vasodilation or smooth muscle relaxation by CO as a physiologically effective agent, or for the treatment of any of acute or chronic systematic hypertension, hyperoxia-induced injury, cancer by the pro-apoptotic effect of CO, transplant rejection, post-operative ileus, post-ischemic organ damage, angina, haemorrhagic shock, penile erectile dysfunction, hepatic cirrhosis, cardiac hypertrophy, heart failure and ulcerative colitis or for treatment in balloon angioplasty or aortic transplantation.

51. A method according to claim 47, which is for treatment of any of acute or chronic systemic hypertension, pulmonary hypertension, transplant rejection, post-operative ileus,

arteriosclerosis, post-ischemic organ damage, myocardial infarction, penile erectile dysfunction, vascular restenosis, hepatic cirrhosis, cardiac hypertrophy, heart failure, chronic anal fissure, internal anal sphincter disease, anorectal disease, and ulcerative colitis or for treatment in balloon angioplasty or aortic transplantation.

52. A method according to any one of claims 47 to 51 wherein the boranocarbonate compound or ion is as defined in any one of claims 5 to 13.

53. A method according to any one of claim 47 to 52 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

54. A method according to any one of claims 47 to 53 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

55. A pharmaceutical composition comprising:

a) a boranocarbonate compound or ion which makes available CO suitable for physiological effect; and

b) a guanylate cyclase stimulant or stabiliser.

56. A composition according to claim 55 wherein the boranocarbonate compound or ion is as defined in any one of claims 5 to 13.

57. A composition according to claim 55 or 56 wherein the guanylate cyclase stimulant or stabilizer is a molecule or ion uncombined with the boranocarbonate compound or ion.

58. A composition according to any one of claims 55 to 57 wherein the guanylate cyclase stimulant or stabilizer is YC-1.

59. A composition according to any one of claims 55 to 58, adapted for one of simultaneous and sequential administration of the boranocarbonate compound or ion and the guanylate cyclase stimulant or stabilizer.

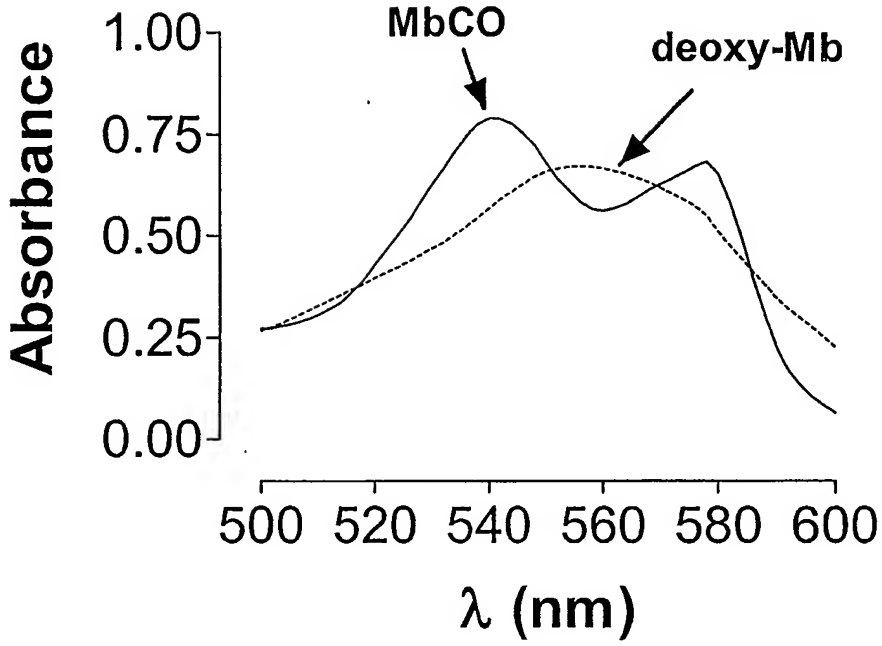

MbCO
deoxy-Mb
Absorbance
1.00
0.75
0.50
0.25
0.00
500 520 540 560 580 600
λ (nm)

Figure 1

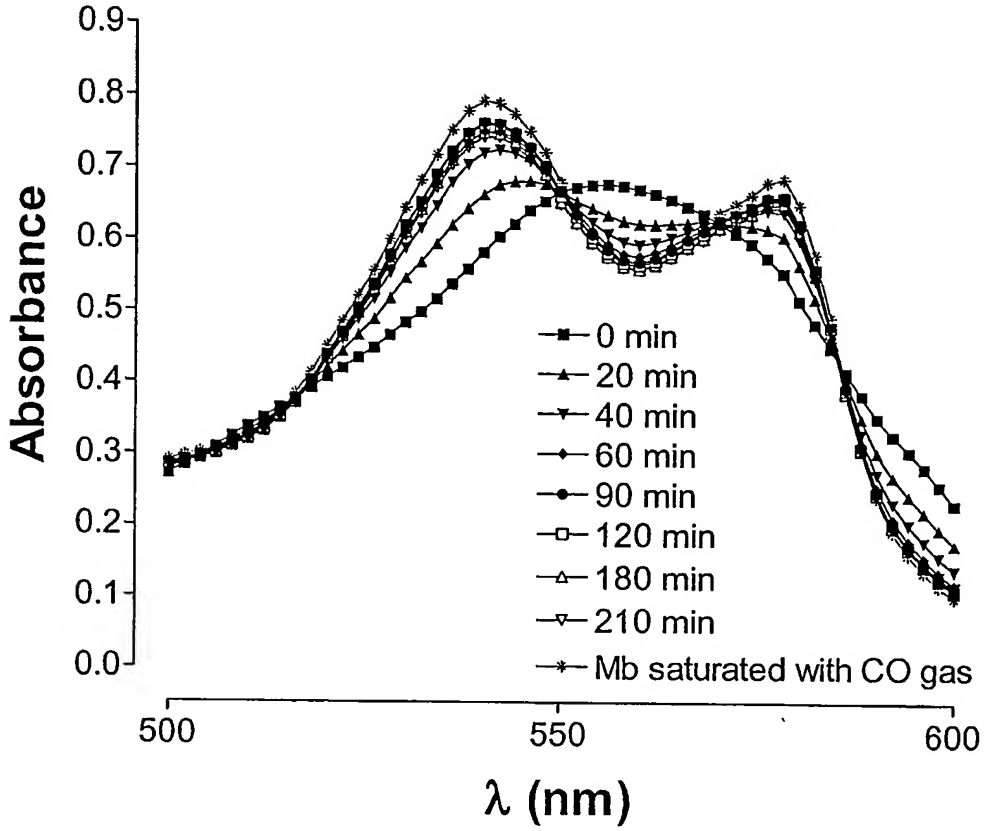

Absorbance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
500
550
600
λ (nm)
0 min
20 min
40 min
60 min
90 min
120 min
180 min
210 min
Mb saturated with CO gas

Figure 2

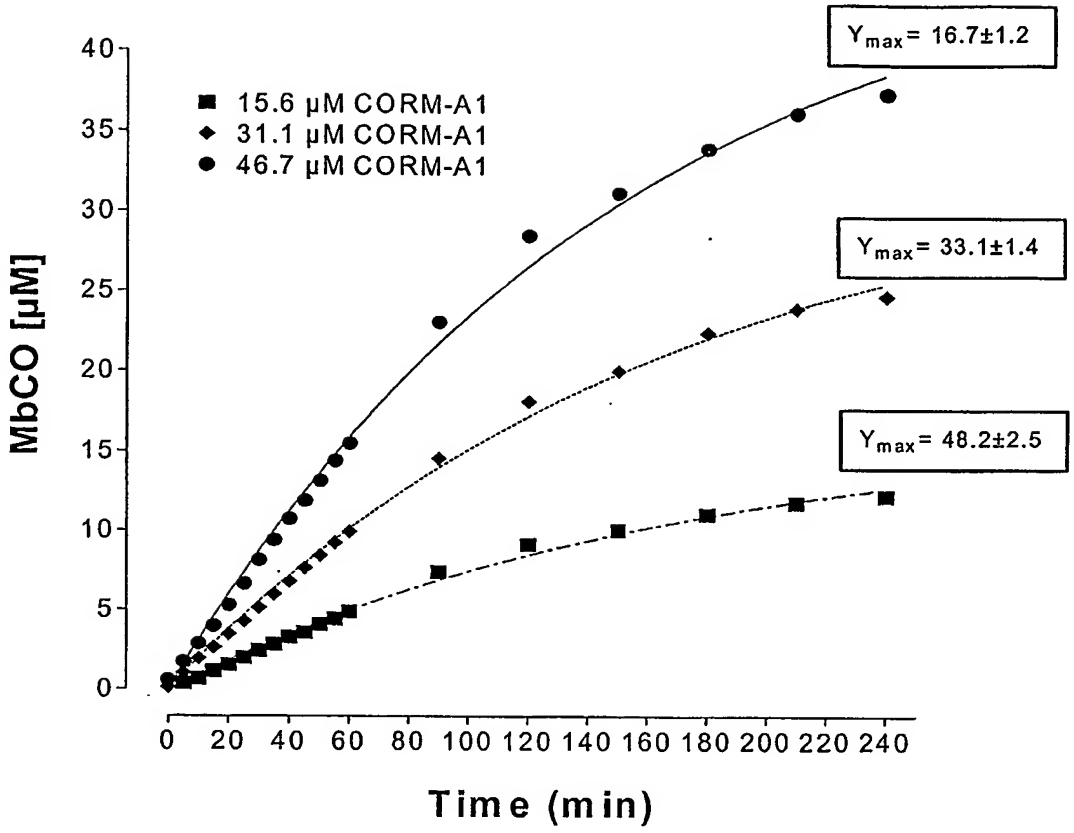

Y max = 16.7±1.2
Y max = 33.1±1.4
Y max = 48.2±2.5
15.6 µM CORM-A1
31.1 µM CORM-A1
46.7 µM CORM-A1
MbCO [µM]
Time (min)
0
5
10
15
20
25
30
35
40
0 20 40 60 80 100 120 140 160 180 200 220 240

Figure 3

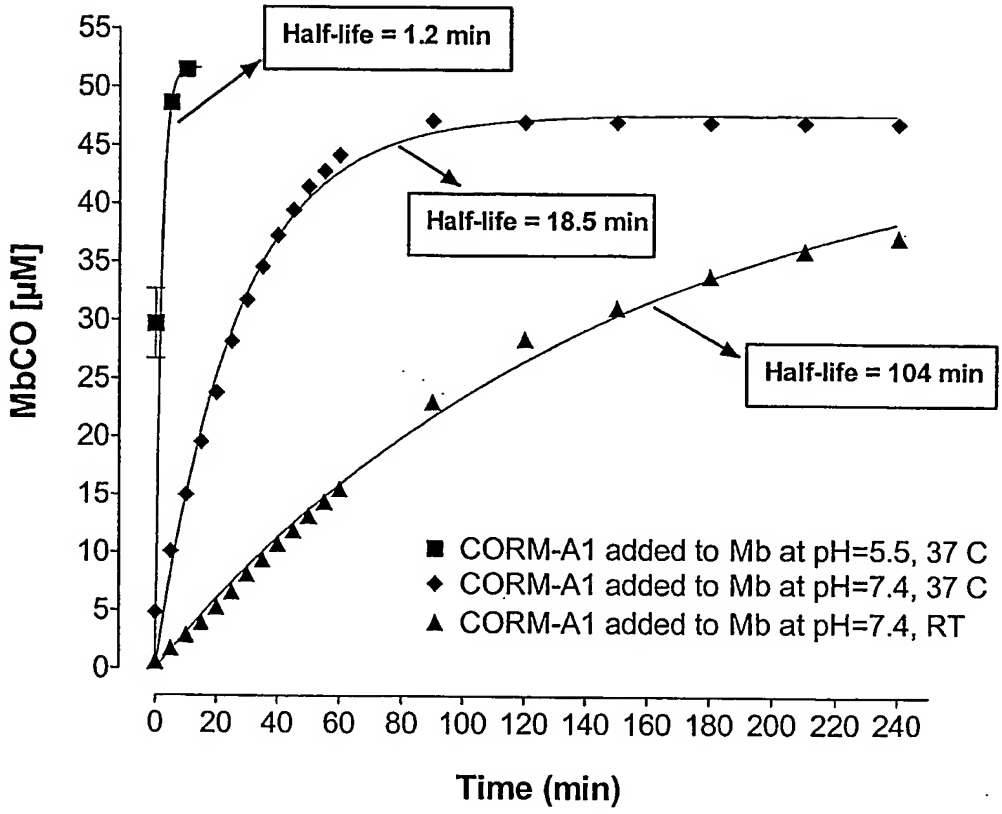

Half-life = 1.2 min
Half-life = 18.5 min
Half-life = 104 min
MbCO [µM]
Time (min)
CORM-A1 added to Mb at pH=5.5, 37 C
CORM-A1 added to Mb at pH=7.4, 37 C
CORM-A1 added to Mb at pH=7.4, RT
0
5
10
15
20
25
30
35
40
45
50
55
0 20 40 60 80 100 120 140 160 180 200 220 240

Figure 4

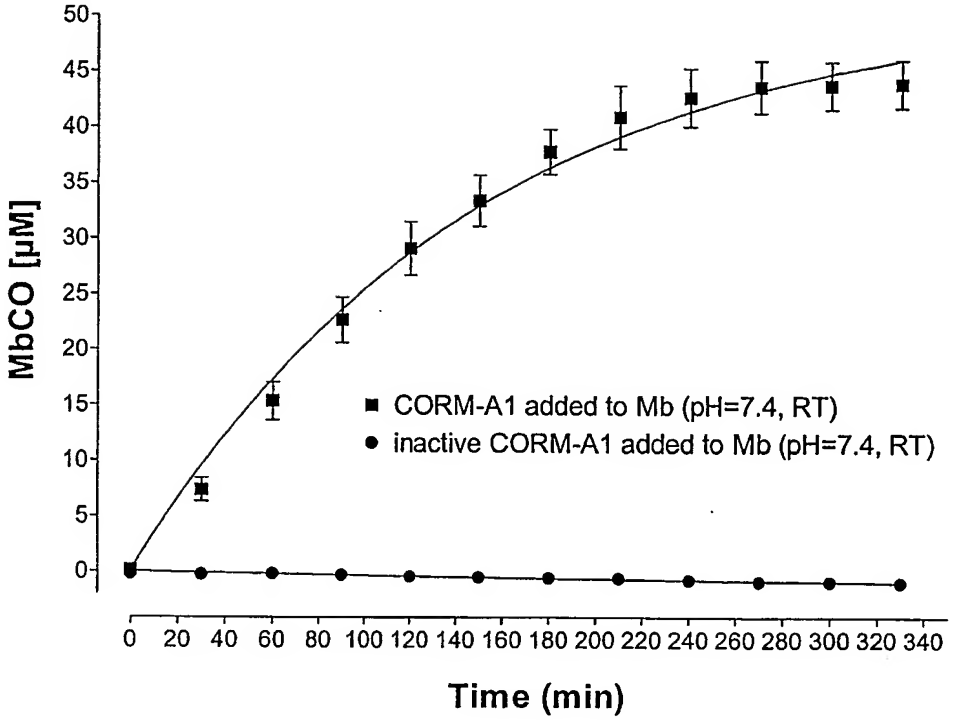

MbCO [µM]
50
45
40
35
30
25
20
15
10
5
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340
Time (min)
CORM-A1 added to Mb (pH=7.4, RT)
inactive CORM-A1 added to Mb (pH=7.4, RT)

Figure 5

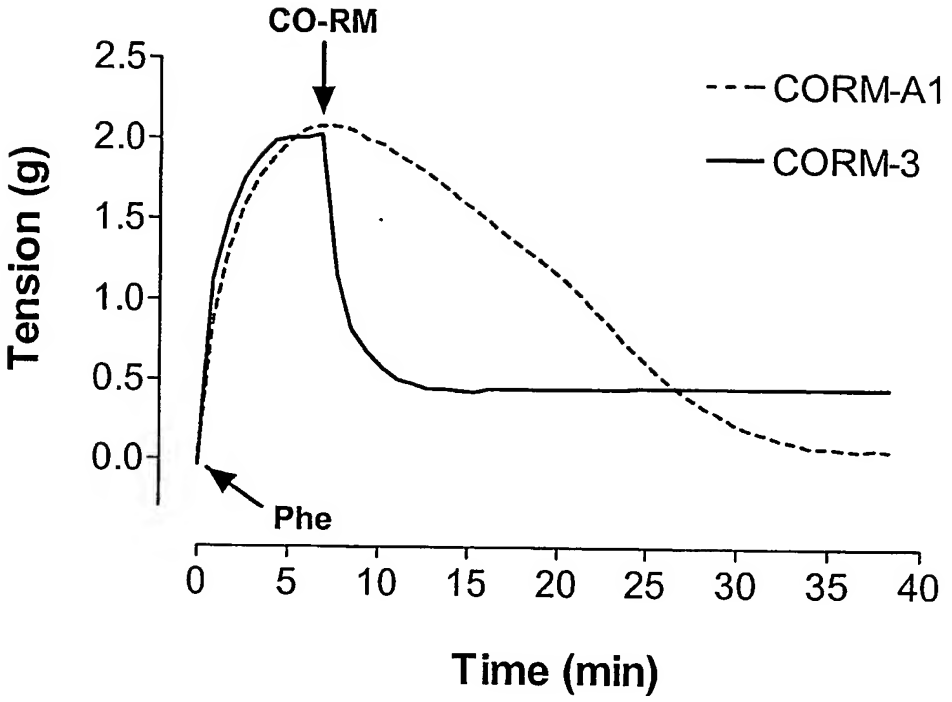

CO-RM
CORM-A1
CORM-3
Tension (g)
2.5
2.0
1.5
1.0
0.5
0.0
Phe
0
5
10
15
20
25
30
35
40
Time (min)

Figure 6

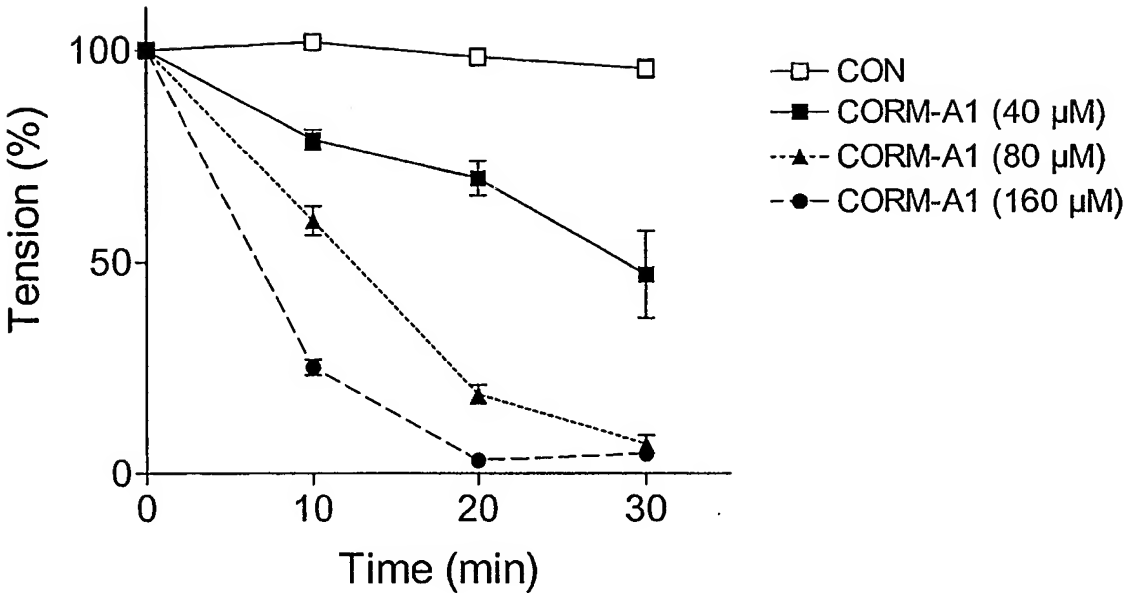

100
50
0
Tension (%)
0
10
20
30
Time (min)
CON
CORM-A1 (40 µM)
CORM-A1 (80 µM)
CORM-A1 (160 µM)

Figure 7

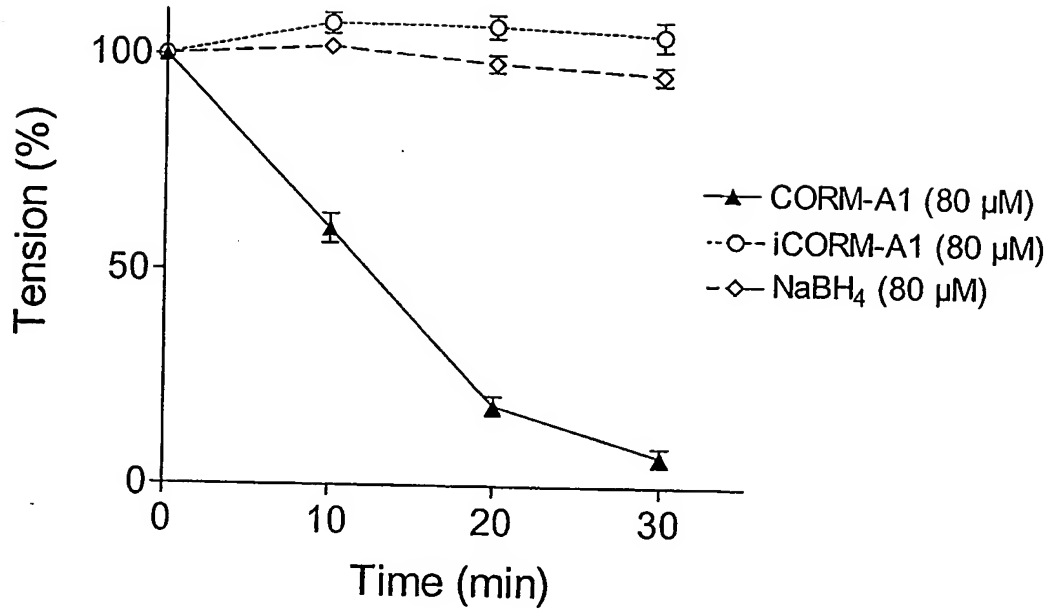
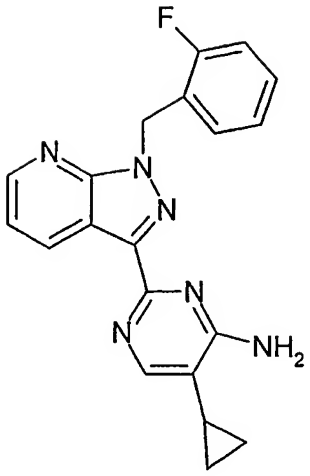
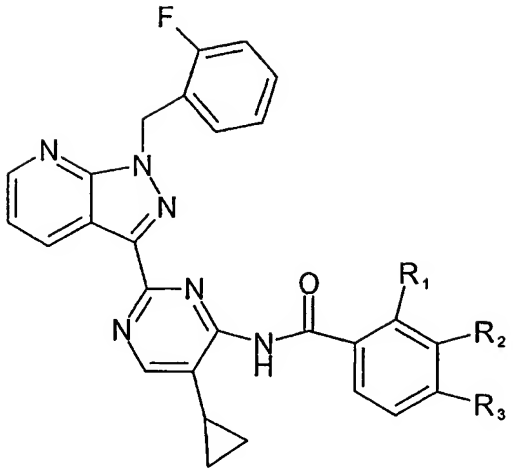

100
50
0
Tension (%)
0
10
20
30
Time (min)
CORM-A1 (80 µM)
iCORM-A1 (80 µM)
NaBH4 (80 µM)

Figure 8



BAY 41-2272



ortho PAL : $R_1 = N_3$ $R_2 = H$ $R_3 = H$
meta PAL : $R_1 = H$ $R_1 = N_3$ $R_3 = H$
para PAL : $R_1 = H$ $R_2 = H$ $R_3 = N_3$

Figure 9

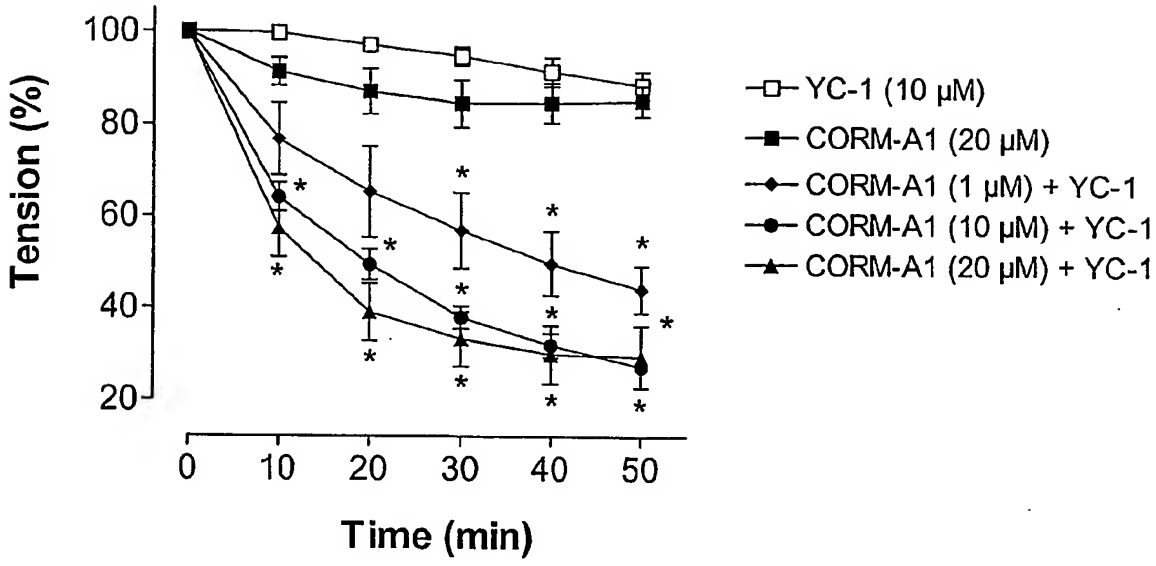

Tension (%)
100
80
60
40
20
0
10
20
30
40
50
Time (min)
YC-1 (10 μM)
CORM-A1 (20 μM)
CORM-A1 (1 μM) + YC-1
CORM-A1 (10 μM) + YC-1
CORM-A1 (20 μM) + YC-1

Figure 10

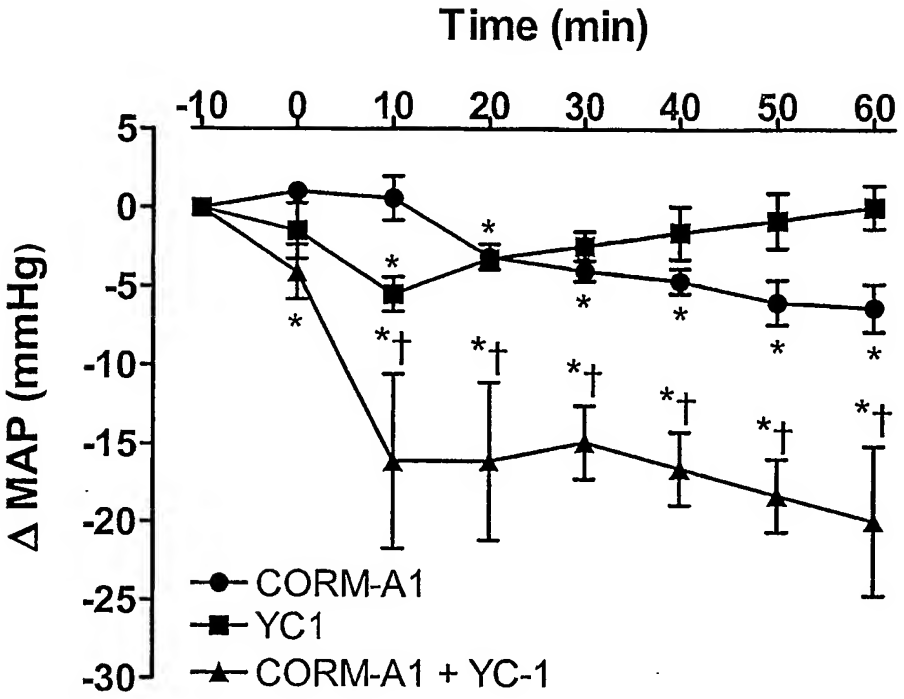

Time (min)
-10
0
10
20
30
40
50
60
5
0
-5
-10
-15
-20
-25
-30
Δ MAP (mmHg)
CORM-A1
YC1
CORM-A1 + YC-1

Figure 11

INTERNATIONAL SEARCH REPORT

GB2004/003365

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A01N1/02 A61K31/416 A61K31/69 A61K45/06 A61P7/04
A61P9/04 A61P9/10 A61P9/12 A61P11/00 A61P29/00
A61P31/00 A61P35/00 A61P41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01N A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 03/066067 A (HAAS WERNER ; ROMAO CARLOS (PT); ROYO BEATRIZ (PT); FERNANDES ANA CRIS) 14 August 2003 (2003-08-14) page 14, paragraph '033! - page 24, paragraph '045! page 25, line 1 - line 17 page 54, paragraph '074! - page 58, paragraph '80!	1,2, 4-12,19, 20,22-30
X	WO 93/05795 A (BORON BIOLOG INC) 1 April 1993 (1993-04-01) cited in the application page 11, paragraph 5 - page 12, paragraph 2 page 19, paragraph 4 - page 22, paragraph 1 page 27, paragraph 2 page 29, paragraph 1	1-13, 18-31,36

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2004	02/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Albrecht, S

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INTERNATIONAL SEARCH REPORT

GB2004/003365

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 632 026 A (ADIR) 4 January 1995 (1995-01-04) page 14, line 44 - line 58 examples 18-22	1,2,4,5, 18-20, 22,23,36
X	WO 94/01413 A (BORON BIOLOG INC ; UNIV DUKE (US)) 20 January 1994 (1994-01-20) page 4, line 8 - line 11 page 8, line 32 - line 33 page 9, lines 13,29,30 page 14, line 20 - line 34 page 17, line 4 - line 21 example 3	1-5, 18-23,36
Y	WO 02/092075 A (UNIV SHEFFIELD ; NORTHWICK PARK INST FOR MEDICA (GB)) 21 November 2002 (2002-11-21) cited in the application page 9, line 14 - line 16 page 12, line 18 - line 27 page 17, line 7 - page 18, line 16	1-59
Y	WO 01/25243 A (ALBERTO ROGER ARIEL ; MALLINCKRODT INC (US)) 12 April 2001 (2001-04-12) the whole document	1-59
X	MILLER M C III ET AL: "The pharmacological activities of the metabolites of N-'(trimethylamineboryl)-carbonyl!-L-phenylalanine methyl ester" METAL-BASED DRUGS 1996 ISRAEL, vol. 3, no. 5, 1996, pages 219-226, XP009038194 ISSN: 0793-0291 page 220; figure 1 page 222, paragraph 4 - paragraph 6 table III	1,2,4,5, 18-20, 22,23,36
Y	STONE JAMES R ET AL: "Synergistic activation of soluble guanylate cyclase by YC-1 and carbon monoxide: Implications for the role of cleavage of the iron-histidine bond during activation by nitric oxide" CHEMISTRY AND BIOLOGY (LONDON), vol. 5, no. 5, May 1998 (1998-05), pages 255-261, XP002300901 ISSN: 1074-5521 the whole document	14-18, 32-36, 39-41, 44-59

INTERNATIONAL SEARCH REPORT

PCT/GB2004/003365

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19-36,47-54
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-36, 47-54 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

GB2004/003365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03066067 A	14-08-2003	CA 2475209 A1	14-08-2003
		WO 03066067 A2	14-08-2003
		US 2004067261 A1	08-04-2004
WO 9305795 A	01-04-1993	EP 0746324 A1	11-12-1996
		JP 7502263 T	09-03-1995
		WO 9305795 A1	01-04-1993
		US 5312816 A	17-05-1994
EP 0632026 A	04-01-1995	FR 2707085 A1	06-01-1995
		AT 160338 T	15-12-1997
		AU 673739 B2	21-11-1996
		AU 6602394 A	12-01-1995
		CA 2127066 A1	31-12-1994
		DE 69406853 D1	02-01-1998
		DE 69406853 T2	18-06-1998
		DK 632026 T3	27-07-1998
		EP 0632026 A1	04-01-1995
		ES 2111871 T3	16-03-1998
		GR 3025951 T3	30-04-1998
		JP 2688175 B2	08-12-1997
		JP 7149727 A	13-06-1995
		NZ 260874 A	26-09-1995
		US 5470864 A	28-11-1995
		US 5585390 A	17-12-1996
		ZA 9404728 A	13-07-1995
WO 9401413 A	20-01-1994	US 5362732 A	08-11-1994
		AU 4658593 A	31-01-1994
		EP 0649411 A1	26-04-1995
		WO 9401413 A1	20-01-1994
		US 5659027 A	19-08-1997
WO 02092075 A	21-11-2002	CA 2447275 A1	21-11-2002
		EP 1399147 A2	24-03-2004
		WO 02092075 A2	21-11-2002
		US 2003064114 A1	03-04-2003
WO 0125243 A	12-04-2001	AU 1134401 A	10-05-2001
		CA 2385927 A1	12-04-2001
		CN 1377360 T	30-10-2002
		CZ 20021118 A3	16-04-2003
		WO 0125243 A1	12-04-2001
		EP 1218385 A1	03-07-2002
		HU 0203138 A2	28-12-2002
		JP 2003511334 T	25-03-2003